

#### REMARKS

A summary of the prior art is believed to be advantageous for a proper appreciation of applicants' invention. Condensed phosphates have been known to prevent the deposition of calcium carbonate from solutions and pyrophosphates have been known to inhibit the precipitation of calcium phosphate when they are added even in low concentrations to solutions of calcium phosphates. Pyrophosphates exhibit a marked effect in vitro on calcium phosphate in concentrations close to the concentrations found in the biological fluids.

Unfortunately it has not been possible to use the pyrophosphates (PP) therapeutically because of the rapid hydrolysis which they undergo when administered both by the oral route and by the systemic route. For the purpose of increasing the resistance to hydrolysis, biphosphonates, that is substances which contain the group P-C-P instead of the group P-O-P have been investigated. The action of the biphosphonates on calcium salts is similar to the action of PP; because even in low concentration, they inhibit the precipitation of calcium phosphate from solutions, block the transformation of amorphous calcium phosphate into the crystalline form, block the aggregation of crystals of hydroxyapatite and retard the degree of dissolution of crystals of hydroxyapatite after the latter have absorbed the biphosphonates from the solutions.

However, pharmacological and clinical studies have shown that several biphosphonates in the treatment of osteopathia

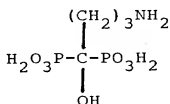
exhibit serious drawbacks with respect to the degree of toxicity in animals and the tolerability or the inducement of side effects in men.

Applicants have investigated biphosphonic acids of general formula I:

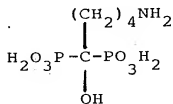


in which R is fluorine or a linear or branched alkyl residue containing between 1 and 5 carbon atoms, substituted by amino groups and R' is hydroxy or fluorine in the treatment of urolithiasis and as inhibitors of the bone reabsorption because they exhibit high activity which is not accompanied by side effects.

Applicants have prepared 4-amino-1-hydroxybutan-1,1-biphosphonic acid, (AHbuBP) of structure hereinbelow:



5-amino-1-hydroxy-pentan-1,1-biphosphonic acid (AHPeBP) of structure



difluoromethanebiphosphonic acid as the sodium salt ( $F_2MBP$ ). Applicants have compared the new products with the known 6-amino-1-hydroxyhexane-1,1-biphosphonic acid (AHEXBP) prepared according to Italian Patent Application No. 19673 A/81 and with the known dichloromethanebiphosphonic acid ( $Cl_2MBP$ ).

Pharmacological tests have been reported in the specification, specifically the effect of the compounds on a culture of skull cells and on the bone reabsorption and the mineralization in vivo and determination of lactate. The data reported show that AHPEBP is the most active in inhibiting the bone reabsorption but manifests some toxicity at a higher dosage. The compound AHEXBP is also active on the reabsorption with a result slightly superior to  $Cl_2MBP$ . A significant difference is with respect to the mineralization because AHEXBP induces strong inhibition of mineralization in the dose of 10 mg of P/kg while AHBUBP has no effect or only a slight effect or only an effect to a very small extent.

These results show that the amino compounds with an odd number of carbon atoms are somewhat toxic but are much more active in inhibiting the bone reabsorption. The compounds with an even number of carbon atoms have an activity slightly superior to  $Cl_2MBP$ . Another significant fact is that AHBUBP does not induce or induces only to a very small extent the inhibition of mineralization at high dosage while AHEXBP exhibits high inhibition. Consequently, AHBUBP appears to be more suitable for use in diseases with an increase reabsorption of bone in

humans. It should be noted that F<sub>2</sub>MBP has no effect on the bone reabsorption or on the bone mineralization.

Clinical studies have also been reported in the specification. The substance AHBUBP has been tested in primary hyperparathyroidism. The results with eight individuals having different degrees of calcemia varying to the extent of 17-11 mg%, were treated. In seven patients who had very elevated calcemia values which had affected the bone not only from the biohumoral but also radiographic point of view, a drastic reduction of calcemia was achieved up to normal values in five of the patients. The decrease of calcemia has been obtained simultaneously with a rapid and parallel decrease of hydroxyprolinuria and decrease of the urinary excretion of calcium. In three cases, the administration of 25 mg/day of the substance for seven days has brought the normalization of calcemia up to the day of surgical intervention of removal of the parathyroidal adenoma. In four other patients treated for shorter period of time, only 3-4 days and with a dose of 4-8 mg/day, the decrease of calcemia has been transitory, with the values of calcemia and the hydroxyprolinuria having a tendency to return to the basal values a few days after the suspension of the drug.

The Examiner's attention is respectfully directed to the specification, page 29, line 1 et seq., where applicants have stated that only in one case with borderline values of calcemia and no symptoms of the bone having been affected, the administration of 4 mg/day of AHBUBP for a period of four days did not cause any variation of the calcemia even if it is

accompanied by a persistent reduction in the calcemia. The comparison of the results obtained in patients with primary hyperparathyroidism using  $\text{Cl}_2\text{MDP}$  reported by Adami et al, Calcif. Tissue Int. 1983 and AHBUBP leads to the conclusion that the latter is 20 - 100 times more active than  $\text{Cl}_2\text{MDP}$ .

In the case of Paget's Disease, three patients were treated with AHBUBP in the dose of 4 mg. in one patient and 0.5mg in the case of the other two patients per day, for a period of 8 and 21 days respectively. In all the three patients, a normalization of the urinary excretion of hydroxyproline was achieved. After 6-8 months, the three patients still exhibit normal values of hydroxyprolinuria and alkaline phosphatemia.

Clinical tests have also been reported in the case of neoplastic hypercalcemia with success, (Page 29, paragraph 3).

The results in the case of neoplastic osteolysis, with six patients treated for a period of two weeks, have shown the normalization of calciuria and a consistent decrease (50-80% of hydroxyprolinuria in five days.

On page 30, line 5 et seq., applicants have stated that AHBUBP inhibits bone reabsorption and is about 100-300 times more active than  $\text{Cl}_2\text{MDP}$ . The two substances differ also with respect to the mechanism of action: the activity on the immunity system appears to be peculiar to AHBUBP. Satisfactory results have been reported in the case of two patients with tumoral osteolysis treated for three days with 50 mg of AHBUBP.

The enclosed declaration of Sergio Rosini reports additional data on the aminodiphosphonates, summarizes the data and compares the data with other diphosphonates. Unquestionably

diphosphonates constitute a new class of agents for the treatment of the diseases characterized by a relative or absolute increase of bone reabsorption. Their administration causes a rapid decrease of hydroxyprolinuria and calciuria, indicative of bone reabsorption, and a slower decrease of alkaline phosphatasemia, which is an index of osteoblastic activity and therefore of bone neoformation.

Dr. Rosini confirms the conclusion of Prof. Fleisch that amino derivatives (amino-propane; amino-butane) are more active than the others and that AHBUBP has an activity more than 100 times higher than that of  $\text{Cl}_2\text{MBP}$  (Clodronate). Also in comparison with other amino-derivatives like APD, AHBUBP has shown an activity 10 times higher. Further the clinical use of APD has been limited by the observation that it causes a decrease in blood lymphocytes and causes fever.

The conclusion that AHBUBP is much more active than Clodronate is based on the studies of clinical testing with tens of patients affected by tumoral osteolysis and mieloma. These studies have shown that doses of 1 or 2.5 mg/die allow a positive response to the drug, as compared with at least 300 mg/die of Clodronate ( $\text{Cl}_2\text{MDP}$ ) required to achieve the same results.

Dr. Rosini also reports (see declaration, page 2, last paragraph) that AHBUBP displays a surprisingly and unforeseeably longer activity than  $\text{Cl}_2\text{MDP}$ . Indeed the substance, in a 5-6 day treatment, causes positive effects which last for weeks or months after treatment. On the other hand,  $\text{Cl}_2\text{MBP}$  must be administered continuously in order to maintain the therapeutic effect.

Dr. Rosini also reports a study with 8 patients affected by multi mieloma (MM) with diffused osteolytic lesions and strong bone pains. Hypercalcemia, calciuria, creatinemia, phosphatemia, serum alkaline phosphatase and hydroxyprolinuria were accurately monitored in all the patients. Treatment with AHBUBP is a dose of 2.5 mg i.v./die for 5 days every third - fourth month, in addition with VCAP polychemotherapy, resulted in a remarkable improvement or disappearance of bone pains within the first 5-6 days from the beginning of the administration. Hydroxyprolinuria, calciuria, hypercalcemia, main symptoms of bone reabsorption, which were high before the treatment, reached normal limits during and after the administration. The progress of osteolytic lesions ceased and sometimes their extension decreased; there is also evidence of the recomposition of pathologic fractures in 2 cases. No side effects were observed.

Dr. Rosini concludes (see declaration page 3, last paragraph) that AHBUBP is effective in reducing the extension of osteolysis of MM, and probably also in delaying the formation of new osteolytic foci. Further he states that the lowering or block of bone absorption, and therefore the remission of pain persists for a long time after the administration of the drug is discontinued. This prolonged action is not observed with other diphosphonates.

Enclosed herein is a copy of a letter dated August 24, 1984 from Prof. Fleisch of the University of Bern to Dr. Rosini. By way of summary, on page 1, last paragraph, he states

that AHBUBP is the most active compound in inhibiting bone resorption and that it exhibits a greater efficiency toxicity margin than AHP<sub>2</sub>BP and AHHeBP. The substance is active even at a dose of 0.001 mg P/kg s.c. (page 1, first paragraph) and ten times as active than the other homologs containing 3,5 and 6 carbon atoms and 100 times more active than Cl<sub>2</sub>MBP. In the same letter, page 2, Dr. Rosini expresses his opinion based on all the comparative studies and reports of other investigators. He states that a small change in the structure produces significant changes in activity and toxicity so that no correlation exists between structure and activity. For instance some compounds which exhibit inhibitory activity of crystal formation in vitro, do not necessarily inhibit bone mineralization in vivo. This lack of correlation is so strong that some investigators even question today whether the P-C-P moiety is essential.

Applicants submit copies of the publications referred to in Prof. Fleisch's letter. The Fleisch 1970 article reports studies on several diphosphonates but the compound AHBuBP is not mentioned. Indeed none of the compounds tested contained an hydroxyl group and only ethane 1-amino 1,1-diphosphonic acid is mentioned.

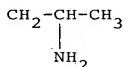
Also the 1970 publication by Russell et al, reports data on several diphosphonates but none of the compounds contained any OH nor NH<sub>a</sub> group.

The Examiner has cited a great number of references. In view of the amendment of the claims, the rejection is believed to be untenable. Blum et al, U.S.P. 4,054,598 describes the use of compounds of formula



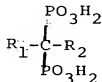


in which X could be



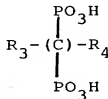
Certainly there is no disclosure of the compound AHBuBP and no disclosure of the superiority of this compound is preventing bone resorption.

Francis, U.S.P. 4,230,700 describes compounds of formula



$\text{R}_1$  could be amino or substituted amino but this reference is totally devoid of any teaching of the compound AHBuBP.

Francis U.S.P. 4,216,211 describes compounds useful in the treatment of hypoxia. The compounds have the formula

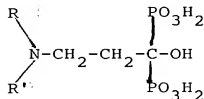


$\text{R}_3$  could be substituted amino, for instance dimethylamino and  $\text{R}_4$  could be OH.

Also Francis U.S.P. 4,330,537 describes the same compounds. Van Duzee, U.S.P. 4,137,309 describes diphosphonic acids in which one R could be substituted amino and the other R could be OH.

Fleisch et al 1970 publication, Ref. R has been discussed hereinabove and it has been shown that it mentions only one compound with an amino group, but no compound containing both OH and an alkylamino group.

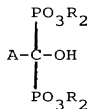
Schmidt-Dunker, U.S.P. 3,962,432 describes compounds of formula



Only compounds with three carbon atoms are shown.

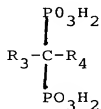
Procter and Gamble, Japanese patent ref. N does not teach the use of the compound AHBuBP but shows only the dichloro compound.

Admittedly Bentzen et al, U.S.P. 4,371,527 describes the use of compounds of formula



and A is an aromatic ring. The same compounds are described in U.S.P. 4,309,364.

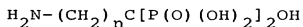
In Baker, U.S.P. 4,330,530, the compounds have formula



in which  $R_3$  could be substituted amino and  $R_4$  could be OH but does not disclose AHBuBP.

The compound used by Bassett ref. 5 is ethane-1-hydroxy 1,1-disodium diphosphonate.

The Examiner has also rejected former Claim 7 because of Chemical Abstracts 96 52503. This abstract mentions the preparation of compounds of formula



in which  $n = 3, 5$

useful as sequestering agents. Clearly the reference is devoid of any teaching of AHBuBP in inhibiting bone reabsorption and the use as sequestering agents is totally different from applicants' use in bone resorption. The Examiner states that the compound of Claim 7 differs merely because of one less methylene group and further states that the compound would be obvious in the absence of a contrary showing. It is submitted that the Examiner is in error. Applicant has shown that AHBuBP is about ten times as active as APD (see Rosini's declaration page 2, second paragraph).

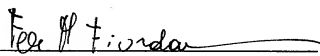
Claim 6 has been cancelled and no discussion is now required.

The table in Rosini's declaration summarizes the effect of several aminobis phosphonates administered for seven days to rats, on the metaphyseal density, which measures the volume of calcified tissue. This table is the same as Table 7 in the specification, page 34. The Examiner's attention is respectfully directed to the specification, page 32, last paragraph to page 36. The compound AHBuBP is about 100 times as active as the lower homolog from propane. On page 33, applicants have concluded that the propane, pentane and hexane compounds

exhibit about the same degree of potency but the butane compound is 10-100 times more active if administered subcutaneously.

It is submitted that applicants have fully complied with the requirement of showing novel and non-obvious results. It was indeed surprising, prior to the present invention, that the compound 4-amino-1-hydroxy..butane-1,1-biphosphonic acid would be so superior to other homologs and analogs in the inhibition of bone reabsorption and for the treatment of urolithiasis. Under the rule of In re Henze, 85 USPQ 261, (CCPA 1950), it is submitted that claim 14 is patentable. A prompt notice of allowance is respectfully urged.

Respectfully submitted,  
BUCKNAM AND ARCHER

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